



12/03/99

UTILITY PATENT APPLICATION TRANSMITTAL

(for new applications under 37 C.F.R. § 1.53(b))

Case No.: F7469(V)
 Applicant: Bernardus Cornelis de BOER; Marnix P. Van AMERONGEN;
 For: FOOD COMPOSITION
 Express Mail Label No.: EE232848796US
 Date Deposited: December 3, 1999
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U.S. PTO
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To: Assistant Commissioner for Patents
 Box: Patent Application
 Washington, D.C. 20231

APPLICATION ELEMENTS

1. ☒ Fee Calculation (Box 13) and Authorization (Triplicate copies of this form are enclosed)
2. ☒ Specification and Claims (17) Total Pages
3. ☐ Formal Drawings () Total Sheets
4. ☒ Unexecuted Declaration
5. ☐ Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary)
 - a. ☐ Computer Readable Copy
 - b. ☐ Paper copy (identical to computer copy)
 - c. ☐ Statement verifying identify of above copies.

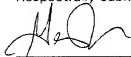
ACCOMPANYING APPLICATION PARTS

6. ☐ Information Disclosure Statement (IDS)/PTO-1449
7. ☐ Copies of IDS citations
8. ☒ Preliminary Amendment
9. ☒ Two (2) Return Receipt Postcards
10. ☐ Certified Copy of Priority Document
11. ☒ The benefit under 35 U.S.C. § 119 is claimed of the filing of European Application 98204419.0 filed December 22, 1998.
12. ☐ Other:
13. ☒ The FILING FEE (including any claims introduced or cancelled by Preliminary Amendment) is calculated below:

CLAIMS					
FOR	NUMBER FILED		NUMBER EXTRA	RATE	BASIC FEE \$760.00
Total Claims	17 - 20			X \$ 18.00	
Independent Claims	1 - 3			X \$ 78.00	
Multiple Claims	<u>Yes</u>	<u>No</u>		X \$260.00	
TOTAL FILING FEE . . .					\$760.00

14. [X] Charge \$760.00 to Deposit Acct. #12-1155. Triplicate copies of this letter are enclosed.
15. [X] The Commissioner is hereby authorized to charge any additional fees, which may be required, including all required fees under
- [X] 37 C.F.R. § 1.16;
 - [X] 37 C.F.R. § 1.17;
 - [X] 37 C.F.R. § 1.18.
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Respectfully submitted,



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Express Mail Label No.: EE232848796US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: de Boer et al.
Deposited: December 3, 1999.
For: FOOD COMPOSITION

Edgewater, New Jersey 07020
December 3, 1999

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

With regard to the above-identified application filed concurrently herewith, please amend the following:

In the Claims:

Claim 4, line 1, please delete the words "or 3".

Claim 6, lines 1, please replace the words "any of claim 5" with --claim 5--

Respectfully submitted,



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FOOD COMPOSITION

Field of the invention

5 The present invention relates to an edible composition containing both (plant) sterols (and/or sterol-containing compounds) as well as carotenoids.

Background of the invention

10 Various plant sterols and plant sterol derived compounds have (when consumed in the right form) a known and well-documented effect on blood cholesterol level. Among these are compounds like β -sitosterol, the hydrogenated form
15 thereof (β -sitostanol), as well as esterified forms thereof. Sterols have been reported to be capable of lowering the level of blood cholesterol (Pollak & Kritchevsky, Monographs in Atherosclerosis, 1981). In particular, the group of 4-desmethylsterols, 4-
20 monomethylsterols, their hydrogenated forms and fatty acid esters have been reported to have a beneficial effect on blood cholesterol level, in particular on LDL-cholesterol.

Compounds belonging to the class of carotenoids are best
25 known for their role as dietary antioxidants, although other potentially protective mechanisms for this group of compounds have been identified e.g. provitamin A activity, role in immunity and cell-cell communication. About 600 carotenoids occur in nature of which the six major ones in
30 the diet are β - carotene, lycopene, lutein, β -

cryptoxanthin, α -carotene and zeaxanthin. Although no thorough, proven, scientific base for claiming a direct relation between cause and effect of the carotenoid intake and disease incidence has been presented thusfar, inverse 15 associations between carotenoid intake or status and disease incidence have been found.

Hence, when it is desired to achieve a lowering of blood cholesterol level, an increased intake of the sterol 10 compounds as defined above may be desired. However, a disadvantage with the use of such compounds is that the absorption of lipophilic compounds, such as β -carotene and lycopene may decrease. It has been reported that diets with an effective amount of cholesterol-lowering (plant) sterol 15 did result in measurable decrease of lipophilic carotenoids in blood.

It is also desired to provide an edible composition that can be incorporated in a single meal which can both supply 20 (plant)sterols and/or (plant)stanols or derivatives, as well as (one or more of) carotenoids. Preferably, in such a way that a net increase in blood carotenoid level is obtained together with an intake of an effective amount of (plant)sterols, (plant)stanols, or derivatives of such 25 compounds. It would be even more preferred if such composition can be part of the main (hot) meal.

WO 98/130233 discloses foodstuffs containing micro-crystalline plant sterols. Preferably, the foodstuff is a 30 spread based on a combination of sweetening agent, a

microcrystalline plant sterol and a suitable berry or fruit. Most of the fruits disclosed do not contain carotenoids in any significant amounts, only apricots do contain β -carotene.

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Summary of the invention

The current invention provides a solution to such problems, by which the blood cholesterol effect of the sterols can be
10 obtained together with a maintained (i.e. no decreased) carotenoid status, preferably with an increased carotenoid status, of carotenoids such as β -carotene, α -carotene, lycopene, lutein, β -cryptoxanthin or mixtures thereof (as measured e.g. by a post-prandial clinical trial by e.g.
15 measuring 1-6 hours after intake). More preferably, the blood cholesterol effect of the sterols can be obtained together with an increased bioavailability of such carotenoids.

20 It has now been found that the above may be achieved by an edible composition comprising (phyto) sterols or derivatives thereof in an amount of at least 0.5 (g) (preferably at least 1.5 (g), more preferably at least 2 (g)) per serving, further comprising at least one
25 carotenoid in an amount of at least 1 (mg) (preferably at least 2 (mg)) per serving, and wherein at least 20% wt of the carotenoid(s) is in another phase than the majority of the (phyto) sterols or derivatives thereof. Preferably, the majority of the carotenoids is in another phase than the
30 majority of the (phyto) sterols or derivatives thereof. As

an example of "another phase", part of the carotenoids may be present in the compositions according to the invention in an extended water phase, e.g. in particulates of vegetable origin, the sterols being present as (part of) a fat phase, e.g. dissolved in the fat, or emulsified in the fat phase. "Majority of ..." in this respect is herein to be understood as "at least 50% by weight of the total amount of ... in the composition per serving". Although the composition according to the invention may contain one (type of) carotenoid (e.g. lycopene) it is preferred that the compositions according to the invention contain more than one (type of) carotenoid.

In the above composition, the carotenoids include preferably β -carotene, lycopene, lutein, β -cryptoxanthin, α -carotene, zeaxanthin or mixtures thereof, as these are among the more hydrophobic carotenoids (with β -carotene and lycopene being most lipophilic). The effect on bioavailability of the present invention is (because of the relative lipophilic character) expected to be most distinct on β -carotene and lycopene. Even more preferred in the compositions according to the invention is the presence of β -carotene and lycopene in weight ratio of β -carotene:lycopene of between 1:20 and 1:0.1, more preferably between 1:10 and 1:0.2. It is also preferred that at least the majority of the carotenoids in the compositions according to the invention are β -carotene, lycopene or mixtures thereof.

A convenient way to achieve the desired amount of carotenoids is present in another phase, is to prepare a composition wherein at least 20% wt (preferably at least 50% wt%) of the total amount of carotenoids is included in the composition in the form of (partly broken up) chromoplasts and/or chloroplasts. Chromoplasts and chloroplasts are microstructures that can be found in cells of vegetable matter by nature. Chromo- and/or chloroplasts of a number of fruit and vegetable species contain carotenoids, in various quantities and compositions. In particular tomatoes are rich in lycopene (predominantly in red tomatoes) and β -carotene (predominantly in orange tomatoes), located to a large extent in chromoplasts. Another good source of β -carotene is carrots.

The above can be achieved conveniently if at least the majority of the carotenoids are present in intact or only partly disrupted chromoplasts or chloroplasts, while at the same time tissue structures of vegetable matter are still relatively intact i.e. recognizable fruit or vegetable particulates, whole cells containing chromo- and/or chloroplasts. This can be achieved by product formulations containing ingredients such as tomatoes (red, yellow or orange), peppers (including sweet peppers in colors red, yellow, orange, red (hot) peppers), water mellon, carrots, pink grapefruit, or other good sources of (particularly but not exclusively) the hydrophobic carotenoids β -carotene and lycopene. It is most preferred if the compositions according to the invention comprise chunks or pieces of said fruit and vegetables.

Preferably, the content of β -carotene and/or lycopene, in the presence of plant sterols (or derivatives thereof), in the compositions according to the invention is such that a 5 significant (i.e. having a statistical significance) absorption of these carotenoids can be shown. This can be determined by a so called post-prandial clinical trial, in which chylomicron-rich fractions - that are isolated from blood samples taken up to 8 or 12 hours after consumption 10 of a meal - are analysed for their carotenoid contents.

Where in this application (plant) sterols are mentioned, phytosterols, phytostanols, or mixtures thereof are meant. Hence, the term (plant) sterols in this application refers 15 to 4-desmethylsterols, 4-monomethylsterols and 4,4'-dimethylsterols, their stanol equivalents, and mixtures thereof in any combination possible. Where in this application, reference is made to (plant) sterol esters, fatty acid esters of such sterols/stanols 20 are meant.

Detailed description of the invention

25 Preferably, the amount of (plant) sterols and carotenoids in the composition is such that levels of carotenoids in the body are increased and the level of (serum) blood cholesterol, and LDL-cholesterol in particular, are decreased, when such composition is ingested regularly, for 30 a period of at least 14 days.

- As various vegetables and fruits like tomatoes are suitable sources of carotenoid-containing chromo- and/or chloroplasts, these are preferred sources for the
- 5 carotenoids. Following this, a composition according to the invention may be a food composition comprising tomatoes, such as e.g. an Italian-style sauce to go with pasta, with added to the sauce (phyto) sterols or derivatives thereof in such an amount that per serving at least 0.5 (g),
- 10 preferably at least 1.5 (g) of (phyto)sterol or derivative thereof is ingested. Such a sauce may be anything from a very smooth, more or less homogeneous mixture, to a thick sauce containing chunks of tomato or other fruit or vegetable, depending on desired end result and processing.
- 15 Even more preferred in the above are levels of at least 2 or even 3 (g) (phyto)sterol or derivative thereof (per serving).

The size and weight of a serving will depend on the type of

20 the food product, but will be about the size/weight of that food product as is usually consumed by the average adult person. For Italian-type tomato sauces for pasta dishes such a serving may be about 150-100 gram.

- 25 Also depending on processing, at least part of the carotenoid-containing chromoplasts and/or chloroplasts is presumably present as a part of plant cells.

Also suitable to provide at least part of the composition

30 according to the invention are (besides various colored

tomatoes): sweet peppers (red, yellow or orange), red (hot) peppers, water mellon, carrots, pink grapefruit, or other fruit or vegetable containing carotenoids. The compositions according to the invention may comprise from 10-99% by weight of these fruit or vegetables. Also, extracts of these fruit and vegetables may be used to provide such carotenoids. In that case, the compositions according to the invention may comprise less than 10% wt of the fruit and vegetables. Furthermore it is possible to use synthetic sources of carotenoids.

In processing the fruit or vegetables to make them suitable for incorporation in the compositions according to the invention a temperature treatment may be applied (besides other processing steps). The carotenoid sources may be heat-treated in order to achieve a partial disruption of the chromo- and chloroplast structures while at the same time recognizable tissue structures are still present.

The fruit or vegetables mentioned may be subjected to pretreatment like washing, cutting, heating, cooking, etcetera, but not extensive homogenization, as this may destroy the chromo- and chloroplasts too much.

An advantage of the above is that the carotenoids that are ingested are carotenoids of natural sources which are abundantly available, such as in tomatoes. Also, the compositions according to the invention may be made in the form of e.g. standard tomato sauces for e.g. Italian-style pasta dishes, which have a universal, natural and healthy

appeal. The compositions according to the invention may also be in the form of (part of) a (hot or cold) paste, puree, ketchup (catsup), soup, juice, sauce or similar product.

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Additionally, the compositions according to the invention are preferably low in fat. (Fat is herein to be understood as to comprise all fatty acid triglycerides such as vegetable oils.) Preferably, the composition should contain 10 less than 10% of fat.

The compositions according to the invention can suitably be used as part of the main (hot) meal. By main meal is meant the meal of the day that provides the largest amount of 15 calories per day, and usually includes hot food, and is taken at the end of the day or during lunchtime.

The invention further relates to a process for preparing the compositions as described above. This can be a process 20 for the preparation of a food product which process comprises (not necessarily in the order given) at least the steps of:

- comminuting of carotenoid-containing fruit or vegetables,
- 25 - heating the carotenoid-containing fruit or vegetables,
- mixing the carotenoid-containing fruit or vegetables with a (plant) sterol or derivative thereof.

Preferably in the above process, said carotenoid-containing 30 fruit- or vegetables comprise tomato, capsicum (red, yellow

or orange), red peppers, water mellon, carrots, pink grapefruit or mixtures thereof. Preferably, the fruit or vegetables to make up the composition according to the invention are chosen such that the weight ratio of (plant) sterols or derivatives thereof : carotenoids in the final composition is between 100:1 and 10000:1. (more preferably between 250:1 and 4000:1).

In the present invention, (phyto) sterols are herein to be understood to comprise also derivatives of these compounds, such as esters. Preferred in this respect are β -sitostanol, β -sitosterol, and esters thereof with e.g. fatty acids (to yield e.g. β -sitosteryl linoleate).

15 Description of figure 1: bioavailability of β -carotene and lycopene in the presence of plantsterols.

Example

20 A post-prandial study was undertaken, in which 20 healthy, adult, human volunteers, 10 male, 10 female (age 30-65) were provided with an early-morning tomato-based meal, after which at regular intervals and for a period of 8 hours blood samples were taken. In the triglyceride-rich fractions (mainly consisting of chylomicrons that carry the digested fat and fat-soluble components from the intestine to the liver) of those blood samples the levels of lycopene, beta-carotene and also vitamin A were determined (using straight-phase HPLC analysis using a nucleosil 30 cyanyl-bonded column). The levels of vitamin A (measured as

retinyl palmitate) and beta-carotene can be taken together as a measure for beta-carotene uptake, as during absorption (or shortly thereafter) beta-carotene is partly converted into vitamin A. The integrated post-prandial response can then be used to estimate the bioavailability of beta-carotene and lycopene from a meal.

The meal consisted of 200 g of cooked macaroni, 130 g of chunky tomato sauce (containing more chunks of tomato flesh than most commercial tomato sauces) made of yellow, orange and super red varieties, 80 g of lean cooked ham (gammon), 20 g of white bread, and 11.6 g of sunflower oil (stripped, i.e. low in antioxidants) and 2.2 g of plantsterols. The chosen level of plantsterols of 2.2 g has been shown in the past to be effective, when consumed daily, in lowering the level of cholesterol in blood.

The plant sterols used have been esterified with fatty acids from sunflower oil. The plantsterol:fatty acid proportion is 3:2. The following plant sterols are present in the formula: sitosterol (45-50%), campesterol (20-30%), stigmasterol (15-25%), and brassicasterol (<5%).

The tomato sauce contained 5.2 mg of lycopene, a usual level, and 2.2 mg of beta-carotene, an unusually high level. The latter was achieved as a result of the presence of a beta-carotene-rich orange tomato variety in the tomato sauce chosen.

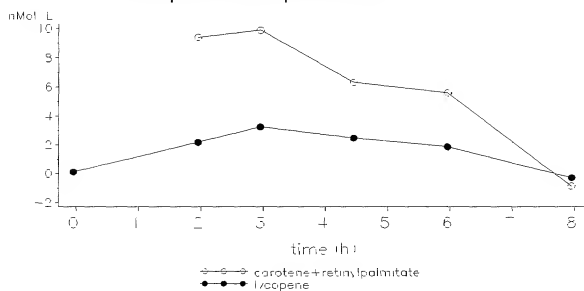
The day preceding the test day the test subjects were asked not to consume foods rich in carotenoids and vitamin A (no vegetables or fruit rich in carotenoids, tomato products, liver products). A deep frozen main meal, low in 5 carotenoids and vitamin A was provided.

On the test day, the meal as above described was consumed between 7.15-8.00 hours. A lunch low in carotenoids, vitamin A, and fat was provided 4.5 hours after the test 10 meal.

Results:

As shown in figure 1, the chunky pasta-sauce based meal with the chosen carotenoid/plant-sterol ratio resulted in a 15 clear post-prandial response of beta-carotene and lycopene. In this graph, the level of carotenoids as measured just before the meal, was taken as zero. This example indicates that this meal, in the presence of 2.2 g of plantsterols, can contribute positively to the carotenoid status in the 20 blood (at least for lycopene and beta-carotene).

Bioavailability of beta-carotene and lycopene in the presence of plantsterols



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Figure 1.

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Claims

1. Edible composition comprising (phyto) sterols or derivatives thereof in an amount of at least 0.5 (g) per serving, further comprising carotenoids in an amount of at least 1 (mg) per serving, and wherein at least 20% wt of the carotenoids is in another phase than the majority of the (phyto) sterols.

2. Composition according to claim 1, wherein the majority of the carotenoids (at least 50% wt) is in another phase than the majority of the (phyto) sterols.

3. Composition according to claim 1, comprising (phyto) sterols in an amount of at least 1.5 (g) per serving.

4. Composition according to claim 1 or 3, comprising carotenoids in an amount of at least 2 (mg).

5. Composition according to claim 1, wherein the carotenoids are selected from the group consisting of α -carotene, β -carotene, lycopene or mixtures thereof.

6. Composition according to any of claim 5, wherein the carotenoids comprises β -carotene and lycopene in a ratio of between 1:20 and 1:0.2.

7. Composition according to claim 1, wherein at least 20% wt of the carotenoids is present in the form of (partly broken up) chromoplasts and/or chloroplasts.

8. Composition according to claim 7, wherein the majority (at least 50% wt) of the carotenoids is present in the form of (partly broken up) chromoplasts and/or chloroplasts.

9. Composition according to claim 8, wherein at least part of the chromoplasts and/or chloroplasts are present as a part of (intact) plant cells.

10. Composition according to claim 1, wherein at least part of the carotenoids are obtained from tomato, (sweet) peppers (red, yellow or orange), red (hot) peppers, water mellon, carrots, pink grapefruit, or other fruit or vegetable containing carotenoids.

11. Composition according to claim 10, wherein at least part of the carotenoid-containing fruit or vegetable has been subjected to extensive heat treatment (temperatures above 70°C).

12. Composition according to claim 1, which comprises (extracts of) processed tomato, capsicum, red peppers, water mellon, carrots, or other fruit or vegetables containing carotenoids, in an amount of 10-99%, based on fresh weight.

13. Composition according to claim 1, in the form of a sauce, paste, puree, ketchup (catsup), soup, juice or similar product.

14. Main meal, comprising the composition according to claim 1.

15. Process for the preparation of a food product which process comprises (not necessarily in the order given) at least the steps of:

- comminuting to pieces of carotenoid-containing fruit or vegetables,
- heating the carotenoid-containing fruit or vegetables,
- mixing the carotenoid-containing fruit or vegetables with a (plant) sterol or derivative thereof.

16. Process according to claim 15, wherein the carotenoid containing fruit or vegetables comprise tomato, (sweet) pepper (red, yellow or orange), red (hot) peppers, water mellow, carrots, pink grapefruit or extracts or mixtures thereof.

17. Process as claimed in claim 15, wherein the amount of carotenoid-containing fruit or vegetable are chosen such that the weight ratio of (plant) sterols or derivatives thereof : carotenoids in the final composition is between 100:1 and 10000:1.

ABSTRACT

The invention relates to an edible compositon containing both (plant) sterols (and/or sterol-containing compounds) as well as carotenoids. Preferably the carotenoids are present in another phase than the (plant) sterols.

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Includes Reference to PCT International Applications)

Attorney Docket No.
F 7469 (V)

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

FOOD COMPOSITION

the specification of which (check only one item below):

- ☐ is attached hereto.
☐ was filed as United States application Serial No. _____ on _____ and was amended on _____ (if applicable)
☐ was filed as PCT international application _____ on _____ and was amended under PCT Article 19 on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. 119
Europe	98204419.0	22 December 1998	YES

I hereby claim the benefit under Title 35, United States Code § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code § 112. I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations § 1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

PRIOR U.S. PROVISIONAL APPLICATION(S) FOR BENEFIT UNDER 35 U.S.C. 119(e)

APPLICATION NUMBER	DATE OF FILING (day, month, year)

I hereby claim the benefit under Title 35, United States Code § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code § 112. I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations § 1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120.

U.S. APPLICATIONS		STATUS (CHECK ONE)			
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED	

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Includes Reference to PCT International Applications)	Attorney Docket No. F 7469 (V)
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PCT APPLICATIONS DESIGNATING THE U.S.				
PCT APPLICATION NO.	PCT FILING DATE	U.S SERIAL NUMBERS ASSIGNED (if any)		

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agents(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (List name and registration number)

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201	SIGNATURE OF INVENTOR 202
DATE	DATE